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SURFACE MODIFICATION FOR BIOCOMPATIBILITY

Contract No. NS 5-2322

Quarterly Progress Report #5

April 30, 1996

The University of Michigan

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Quarterly Progress to:	National Institute of Health
Contract Monitor:	William Heetderks, Ph.D.
Research Contract	"Surface Modification for Biocompatibility"
Contract No.	NS 5-2322
Principal Investigators:	David C. Martin and K. Sue O'Shea
Date:	April 30, 1996

Overview

This report is a summary of our activity in the fifth quarter of the contract, corresponding to the first quarter of 1996. In this fifth period of our activity we have continued to refine our processing and characterization schemes. We have also obtained information about the biological response of the protein coated suture during *in vivo* implantations. This report provides an overview of the major results to date and discusses our plans for the future. We have been working to evaluate (1) protein polymer film deposition and morphology, (2) bioactivity of protein polymer films *in vitro*, and (3) bioactivity and stability of protein polymer films *in vivo*. We also describe our efforts to discuss our work in (4) external communications with the scientific community.

1. Protein Film Deposition, Morphology, and Device Characterization

Progress:

We have obtained information about the mechanical properties of the deposited protein films. Figure 1 shows an SEM image of a deformed, electrospun protein film indicating the fine fibrillar character of the microstructure. The best adhesion appears to occur between the first layers of the deposited filaments and the solid surface. Initial results indicate that the mechanical strength of the electrospun SLPF film is at least 43 kPa. Further efforts to provide information about the them mechanical properties of the films are in progress.

In order to examine probe morphology, and in anticipation of probe trials *in vivo*, we have been working to develop techniques for sectioning and characterizing the silicon probes themselves. The ultramicrotomy of silicon probes embedded in epoxy proved to be problematic even with a diamond knife due to the stiff, brittle character of the substrate leading to fracture during cutting. We were successful, however, in viewing the cross section of a micromachined probe by mounting the sample in a room temperature curing resin followed by metallographic polishing and examination in a scanning electron microscope. The distribution and size of the dielectric layers and polysilicon raceways could be readily identified and dimensions characterized.

We have also recently developed an alternative approach to creating porous protein films by using a gas liberation process. The protein polymer solution in formic acid can be cast as a film onto a solid substrate, and then a coating of sodium bicarbonate ($NaCO_3H$: baking soda) in water is applied. The sodium bicarbonate and organic acid react vigorously to liberate carbon dioxide, which causes the rapid formation of large, fairly uniform pores throughout the structure of the polymer film. The figures enclosed show porous SLPF

films created with (1) ~10% porosity and a mean pore size of 0.1 μm^2, and (2) ~30% porosity and a mean pore size of 1 μm^2. The size and extent of the porosity can be controlled by variations in the concentration of protein in the formic acid and sodium bicarbonate in the water. An additional advantage of this approach is that the volatility of the escaping gas naturally leads to an assymmetric film structure, since there is no gas diffusion into the silicon substrate. The result is a film with a more porous external surface, which is consistent with our interest in maintaining a dense film near the solid surface and loose, open network near tissue. The process generates a sodium formate salt byproduct, which we have so far found can be readily removed by washing in water. Future efforts will provide more detailed information about the morphology of these films, and will relate the bioactivity and physical properties to film microstructure.

Plans:

Our collaborators in the EECS department (D. Anderson, J. Weiland) have recently acquired their own impedence spectroscopy hardware which now makes it possible for us to rapidly obtain detailed information about the electrical performance of the device as a function of deposited film composition and morphology. We are currently evaluating the electrical performance of coated probes as a function of protein film thickness and microstructure.

We have recently set up an apparatus for measuring the surface energies of solid substrates by quantitative analysis of the contact angles of deposited liquid droplets, and intend to use this approach to measure the change in energetics of the silicon surface as a function of deposited protein.

We are continuing our systematic studies of probe electrical performance as a function of coating thickness and morphology. We are also continuing detailed studies of protein film morphology by low voltage scanning electron microscopy, atomic force microscopy, and transmission electron microscopy. We are initiating a study of the microstructure of the probe by TEM, particularly near the iridium site before and after activation.

2. Bioactivity of Protein Polymer Films *in vitro*

Progress:

Our recent efforts have concentrated on the biological activity of patterned substrates. Our patterning efforts continue to show success and interesting results. We have now shown that cell adhesion is preferential to the patterned SLPF squares at short time periods. Figure 1 shows glial cells attached to a square of SLPF deposited on the surface of a silicon wafer. The fidelity of the pattern is most pronounced after four hours. After longer times, the cells apparently develop the ability to adhere to the unmodified silicon surfaces as well.

Future Work:

We are currently developing coatings with alternating stripes and patches of SLPF (fibronectin) and SLPL (laminin) protein polymers, with the intent of directing cell attachment. Our hypothesis is that the SLPF domains will be preferential toward the attachment of glial cells, while SLPL will be favorable toward neurons. SELP patches could also be used to resist cellular adhesion. Examinations of the variation of cellular adhesion with time will provide information about the dynamics of this process. It will

then be possible to imbibe the polymer coatings will small molecules of interest for promoting a specific biological response.

3. Bioactivity of Protein Polymer Films *in vivo*

Progress:

Polypropylene suture (~50 micron diameter) was coated with the following materials and implanted in the Guinea Pig CNS:

1. no coating (control)
2. SLPF coated
3. SLPL coated
4. SLPF/Schwann cells
5. SLPF coated and exposed to CSF
6. SELP coated

The three month implants have now all been examined histologically. There are no dramatic negative responses for any of the implanted samples. There are subtle indications of changes in the first three to four cell layers near the implant consistent with the formation of a small rim of astroglia. Neurons appeared morphologically normal, although there was some re-orienting of processes toward the SLPL coated implants. Quantitative evaluations of this data are underway.

Some TEM data has also been obtained from these samples, which we are working to corroborate. It will be necessary to associate any asymmetry in tissue response with the presence of the protein coating. Preliminary results have revealed the sensitivity of the thermoplastic polypropylene suture to irradiation in the electric beam using the Kresge microscopy facilities. These data are currently being complemented with the Materials Science electron optics facilites at the North Campus Electron Microbeam Analysis Laboratory (EMAL).

Plans:

The nine-month embedded implants will be available for examination in August of this year. The EECS and Kresge group have also been making progress in the development of techniques for probe performance evaluation by confocal microscopy, and we hope to work with them to answer questions of mutual interest.

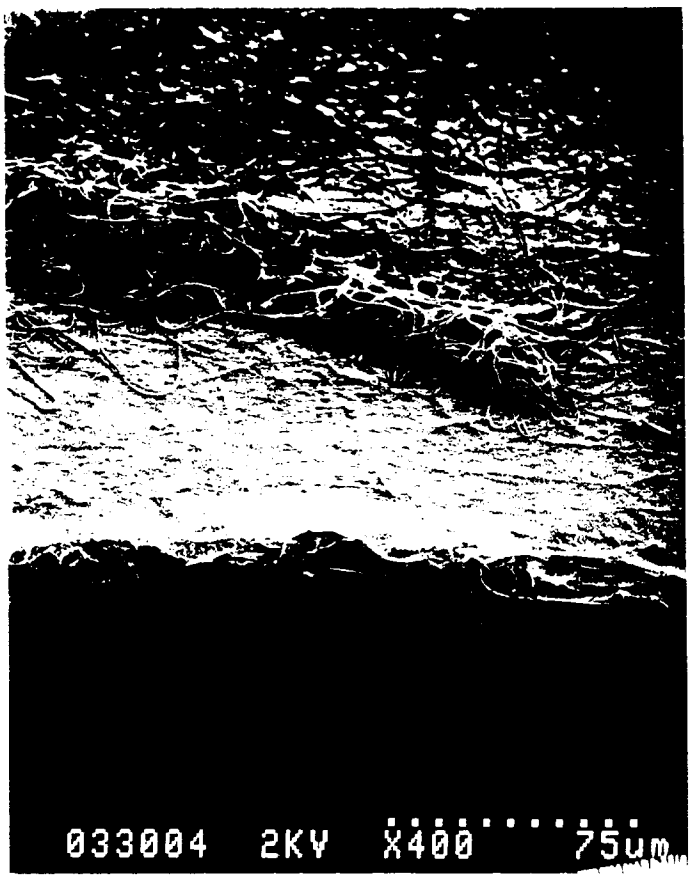
4. External Communications

The invited review paper on *Processing and Characterization of Silk-like Protein Polymers*, by David C. Martin, J. Philip Anderson, Chris Buchko, and Tao Jiang, which is scheduled to appear in a special volume on *Protein Polymer Materials*, edited by Kevin McGrath and David Kaplan of the U. S. Army Natick RD&E Center, has been completed and submitted for review.

An abstract for the Society of Neuroscience has been written titled "Biocompatibility of CNS Implants Coated with Silk Polymers Containing Elastin Fibronectin or Laminin Cell Binding Motifs" A copy is included with this report.

An abstract titled "Length-Scale Dependent Surface Roughness Measurements of Bioactive Polymer Thin Films Using Scanning Probe Microscopy" by was submitted for the Microscopy Society of America meeting in Minneapolis, MN for August, 1996.

CJ Buchko
4/30/96



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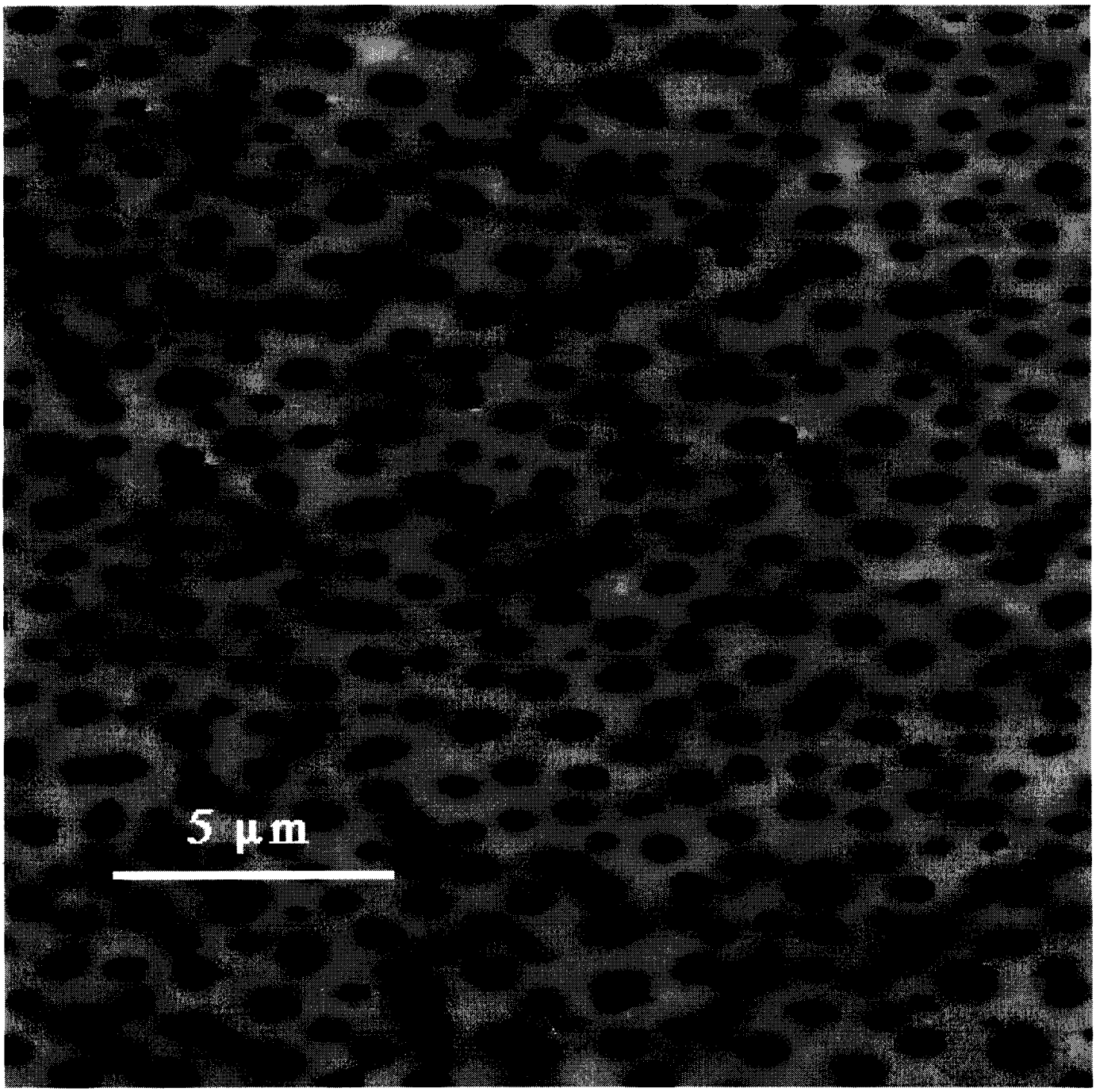

~10% Porosity

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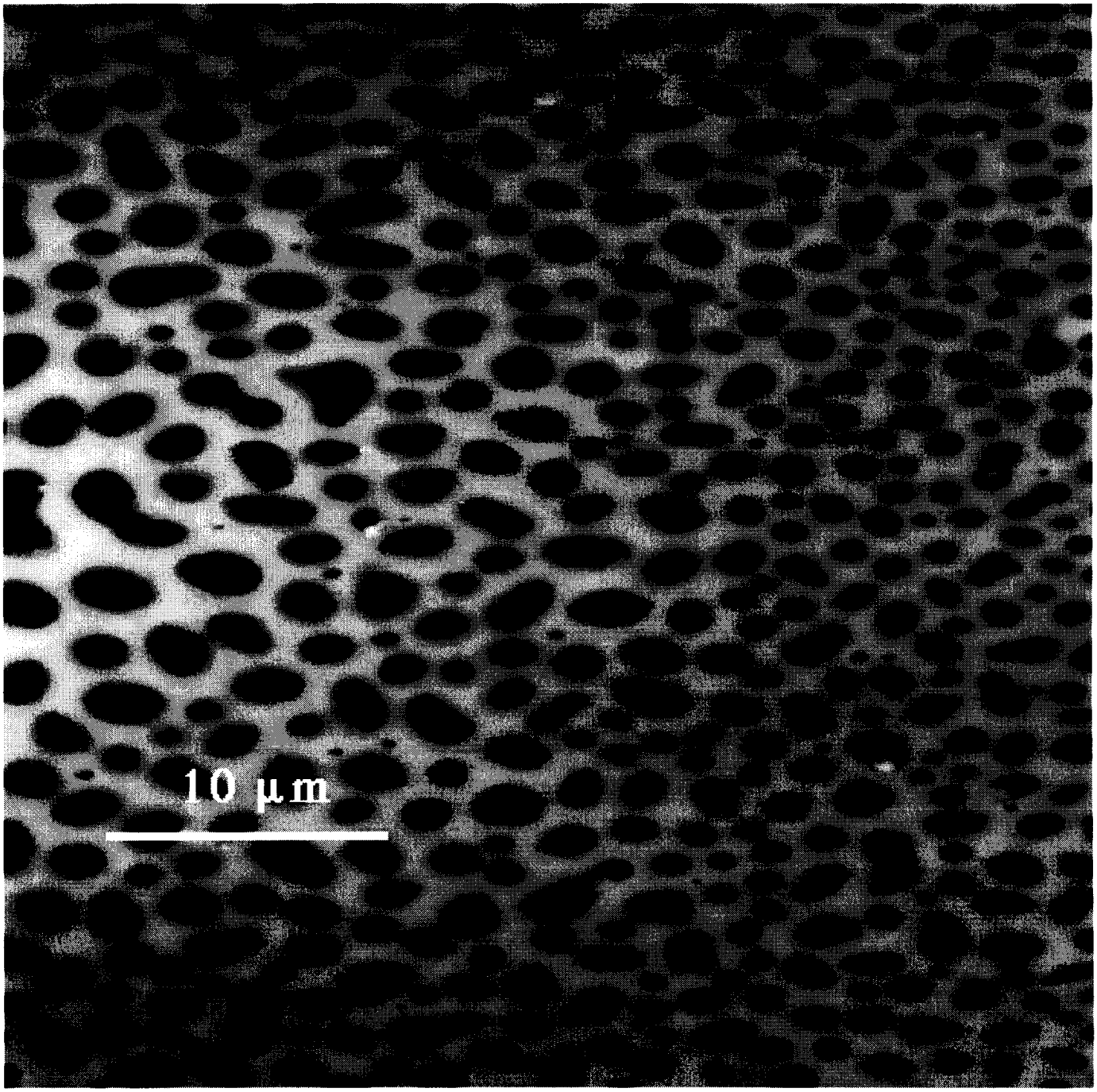
CJB



~ 30% Porosity

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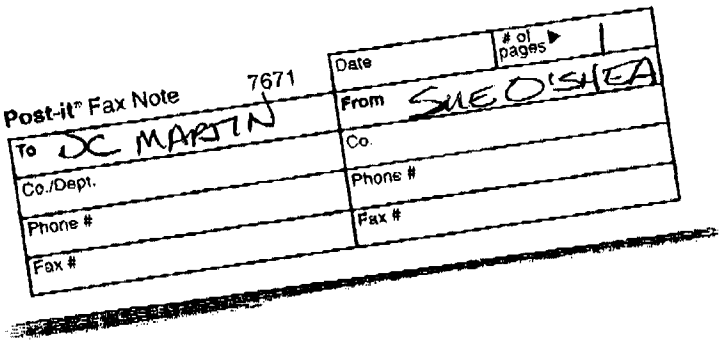

BIOCOMPATIBILITY OF CNS IMPLANTS COATED WITH SILK POLYMERS CONTAINING ELASTIN, FIBRONECTIN, OR LAMININ CELL BINDING MOTIFS. K.S. O'Shea[*1], C. Buchko[2], Y. Shen[2], R.A. Altschuler[3], P.Finger[3], J.A. Wiler[3], J.Cappello[4], and D.C. Martin[2]. Depts. of Anatomy & Cell Biology[1], Materials Science and Engineering[2], the Kresge Hearing Research Institute[3], Univ of Michigan, Ann Arbor, MI, and Protein Polymer Technologies[4], San Diego, CA.

In order to test coatings designed to improve cell-implant interaction, 6-0 polypropylene sutures were coated with protein polymers produced in *E. coli* which were engineered to produce repeating silk-like domains (GAGAGS) combined with extracellular matrix - cell binding motifs, e.g., the RGD sequence from fibronectin (SLPF), IKVAV from the laminin alpha chain (SLPL), or an elastin repeat (VPGVG; SELP). Suture coated with polymer, uncoated suture, or suture coated with SLPF and a layer of mouse Schwann cells were placed in guinea pig cortex and cell reaction to implants assessed at 3 weeks and 12 weeks in Epon sections. At both time points assessed, there was little tissue reaction to the implants, with a small rim of astroglia present at the interface. Neurons appeared morphologically normal, although there was some re-orienting of processes toward the SLPL coated implants. Current investigations are in progress to extend these observations to nine months, with the long term goal of improving implant stability and reducing glial reaction to CNS implants. Supported by NIH NINDS Contract No. NO1-NS-5-2322, and NIH NCRR P41-RR09754.

LENGTH-SCALE DEPENDENT SURFACE ROUGHNESS MEASUREMENTS OF BIOACTIVE POLYMER THIN FILMS USING SCANNING PROBE MICROSCOPY

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The development of implantable biomedical devices requires active biological interfaces that minimize the body's immune response. We have identified candidate protein polymer coatings for biomedical applications and methods of processing these coatings that promote a favorable *in vitro* biological performance.[1] However, the chronic implantation of biomedical devices, specifically the neural prosthetic devices developed at the University of Michigan,[2] places additional requirements on the polymer coatings. Among these constraints are the efficient transport of neuronal signals from tissue to the device and the prevention of physical displacement of the device through tissue. To meet these potentially conflicting goals, we are developing discontinuous, porous polymer coatings that are rough at biologically-relevant length scales.

In order to quantify the surface morphology of these thin polymer films, which are typically 0.02 μm to 5.0 μm thick, we employ a number of complementary techniques, including scanning probe microscopy (SPM), scanning electron microscopy (SEM), light optical microscopy (LOM), and transmission electron microscopy (TEM). Among these techniques, SPM provides convenient information about the morphology of these polymer films at the biologically-relevant length scales of 1 μm to 100 μm. SPM can be used to determine characteristic length scales and identify related scaling regimes.[3] The instrument used for this work was a Digital Instruments NanoScope III with Multimode Head.

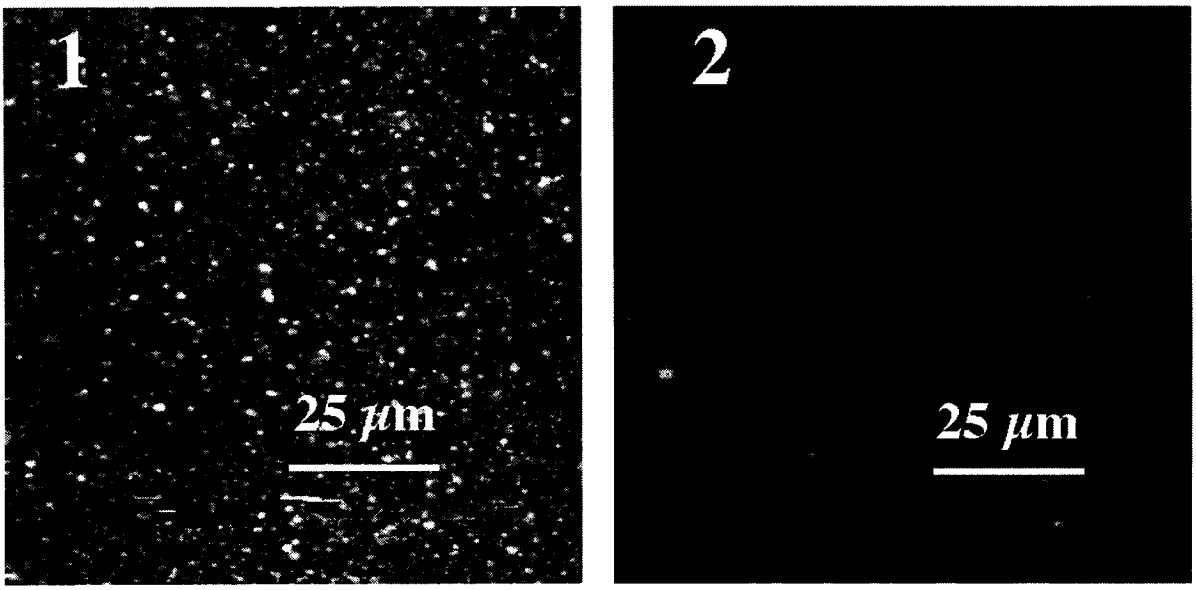
Polymer coatings are electrostatically deposited on substrates to produce discontinuous films that can have a variety of morphologies. Figures 1 and 2 show different types of a beaded coating morphology characteristic of electrostatic deposition from a dilute polymer solution.[1] The wide distribution of polymer bead sizes and the random nature of the surface coverage can be seen in both of these figures. The polymer coating in Figure 1 (Sample A) is discontinuous enough to reveal the silicon surface beneath it while the coating in Figure 2 (Sample B) is more continuous. The roughness of these coatings depends upon the length scale at which the roughness was measured. Power spectral density (PSD) measurements can be used to quantify the length-scale dependence of surface roughness.

The PSD of these surfaces (Figure 3) indicates that Sample A is rougher at length scales below 2 μm while Sample B is rougher at length scales above 2 μm. The change in slope that occurs in each of the samples describes characteristic dimensions of the polymer beads on the surface. The sharpness of the transition from one slope regime to another is related to the spread in sizes of the beads on the surface.

The PSD provides a quantitative means for determining characteristic morphological parameters such as discontinuity, porosity, and roughness. This information can be used to optimize the biological, electrical and mechanical properties of the polymer coatings.[4]

References:
1. C. J. Buchko et al., *Thin Films and Surface for Bioactivity and Biomedical Applications,* ed. C.M. Cotell, S.M. Gorbatkin, G. Grobe, and A.E. Meyer (Mater. Res. Soc. Proc. Pittsburgh, PA, 1995)
2. Center for Neural Communication Technology, http://www.engin.umich.edu/center/cnct/
3. G. Beaucage, D.W. Schaefer, *Journal of Non-Crystalline Solids,* 172-174, (1994) 797-805.
4. This work was supported by NIH Contract N01-NS-52322, the Whitaker Foundation, and Protein Polymer Technologies, Inc.

FIGURE B



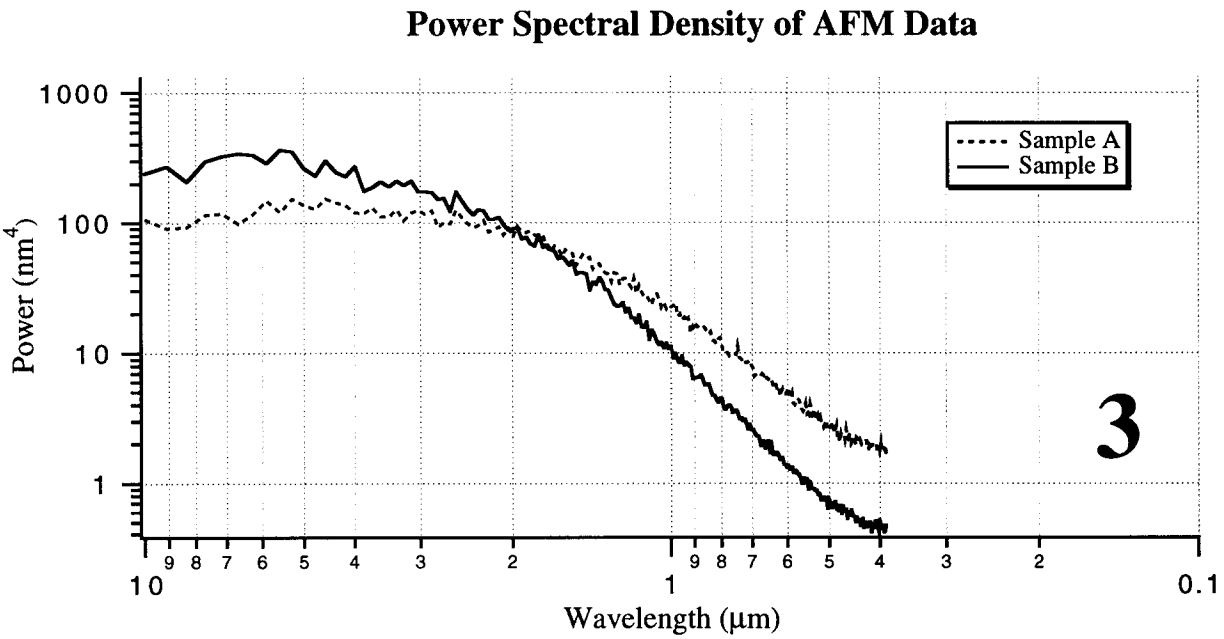


FIG. 1 Contact mode AFM scan of SLPF beads electrodeposited on a silicon surface to form a thin, discontinuous coating (Sample A).

FIG. 2 Contact mode AFM scan of SLPF beads electrodeposited on a silicon surface to form a thicker, more continuous coating (Sample B).

FIG. 3 Power spectral densities of Figures 1 and 2. Sample A is rougher at low wavelengths while Sample B is rougher at higher wavelengths.